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Skeletal Muscle Adaptation in Response to Supervised Exercise Training for Intermittent Claudication

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WHAT THIS PAPER ADDS

- This is the first study in which myosin protein expression has been been used, as apposed to immunohistochemistry, to seek evidence of skeletal muscle adaptation in response to supervised exercise training for claudication.

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ABSTRACT

Objectives: There is evidence that the improvement following supervised exercise for claudication results from skeletal muscle adaptation. The myosin heavy chain (MHC) determines muscle fibre type and therefore efficiency. Immunohistochemical analysis has failed to take account of hybrid MHC expression within myofibres. This study sought evidence of differential MHC protein expression following supervised exercise for claudication.

Design: 38 claudicants were recruited. Subjects undertook a three-month supervised exercise programme. Controls were patients awaiting angioplasty for claudication.

Materials and methods: Subjects underwent paired gastrocnemius biopsy. Relative expression of MHC proteins was determined by SDS-PAGE electrophoresis. Non-parametric data is presented as median with the inter-quartile range and parametric as the mean ± standard deviation.

Results: Upon completion of the exercise programme there was a 94% increase (124 (106–145) to 241 (193–265) metres, $p = 0.002$) in maximum walking distance, which was not evident in the control group. An 11.1% ($p = 0.02$) increase in MHC I expression was observed in the exercise but not the control group (34.3% ± 6.8 to 45.4% ± 4.4). There was a positive correlation between the change in MHC I expression and the improvement in claudication distance ($r = 0.69$, $p < 0.05$).

Conclusions: Supervised exercise training for claudication results in an increase in the proportion of MHC type I expression within the symptomatic gastrocnemius muscle.

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Introduction

Intermittent claudication is a common problem in an ageing population, with some reports that 20% of the over 75's are affected.[1] There has been little change in the treatment of claudication since the advent of endoluminal angioplasty in 1964.[2] Interventional treatments for claudication are not without risk and in addition there are concerns about the longevity of benefit following endovascular therapy.[3,4] Whilst all patients should have their risk factors addressed in view of the associated cardiovascular mortality and morbidity,[5] pharmacological agents have only been shown to produce modest benefits when treating symptoms of claudication.[6] The mainstay of treatment for intermittent claudication has therefore been risk factor control and exercise.[7] Supervised exercise training has been shown to have significant symptomatic benefits in claudication producing a 108% in treadmill assessed pain free walking,[8,9] but in view of the significant co-morbidity associated with claudication[5] not all patients are able to participate and there remains poor provision in the UK.[10]

The mechanisms for this symptomatic improvement witnessed as a result of exercise training remain unclear. There is no

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reproducible evidence to support the hypothesis that supervised exercise induces the development of collateral blood vessels, resulting in enhanced whole limb blood flow[9] and whilst it has been hypothesised that exercise training may result in re-distribution of blood flow within the symptomatic limb, there is only one study to support this, with no corroborating data published since 1969.[11]

The observation that haemodynamic improvements following re-vascularisation[12] are not accompanied by a comparable functional improvement has led to the concept that in addition to the limitation of blood flow resulting in claudication, there may also be an acquired metabolic defect or "metabolic myopathy".[13] It has been hypothesised that supervised exercise training may reverse this metabolic myopathy, a hypothesis which is supported[14] by reports of reduced accumulation of the mitochondrial buffers, the acyl-carnitines, as a result of supervised exercise training.

One of the key determinants of skeletal muscle metabolism is the myosin heavy chain (MHC) of which three isoforms are expressed in mature skeletal muscle; MHC I, IIa and IIb. Slow twitch muscle fibres (type I) are composed of MHC I and are coupled to efficient oxidative metabolism. In contrast, fast twitch muscle fibres composed of MHC IIb are glycolytic and rapidly switch to anaerobic metabolism under stress resulting in the accumulation of lactate.

An increase in the proportion of MHC type I is potentially beneficial for claudicants, increasing oxidative capacity and reducing the rapid switch to anaerobic glycolysis associated with the faster glycolytic fibres (MHC IIb).

Immunohistochemistry has been used to seek evidence of skeletal muscle adaptation resulting from a 6-week programme of exercise training in claudicants[14] and no change in the type I fibre area was observed. It has however since become apparent that muscle fibres are actually hybrids expressing more than one myosin heavy chain along their length and this diversity is more prominent in ageing skeletal muscle.[15] As a result, traditional mATPase immunohistochemistry may underestimate any potential muscle adaptation. One small study in patients with heart failure using SDS-PAGE electrophoresis to determine MHC protein expression has reported a significant 12.6% increase in MHC I expression in response to a supervised resistance cycling training programme.[16]

Methods

The initial study design of a randomised control trial was refused by the regional research ethics committee, who determined that it was unethical given the evidence for its effectivity not to offer all claudicants supervised exercise training. Ethical approval was therefore obtained for a non-randomised study in which patients awaiting angioplasty for claudication were recruited to the control group.

Using Keteyians' data in which SDS-PAGE electrophoresis was used to study patients with heart failure, a power calculation was undertaken. A sample size of 26 paired biopsies would have a 90% power to detect a 10% difference in means at the $p = 0.05$ significance level.

Thirty-eight subjects with stable claudication were recruited from vascular outpatient clinics at the Bristol Royal Infirmary and informed consent was obtained. Twenty-seven subjects were recruited to a 12-week supervised exercise programme for claudication. Paired Bergstrom needle skeletal muscle biopsies were taken from the symptomatic gastrocnemius muscle at recruitment and upon completion of the study. A further 11 subjects with symptomatic calf claudication, who were awaiting endovascular re-vascularisation, were recruited to a control group and paired skeletal muscle biopsies were also taken at recruitment and following a period of exercise advice, prior to re-vascularisation. The controls were matched as closely to the exercise group as possible for age, sex, ABPI, walking distance, and the distribution of disease. The control group were advised to exercise at least three times weekly for a minimum of 30 min, walking to the point at which claudication becomes intolerable.

All subjects had at least a six-month history of stable symptomatic calf muscle claudication, which was induced on treadmill testing at 2.5 kph and 10° of incline.

Patients who had previously had endovascular or surgical intervention or who had attended a supervised exercise programme for claudication were excluded. All subjects were already receiving an anti-platelet agent and a statin and subjects treated with a phosphodiesterase inhibitor for claudication were excluded.

All subjects underwent an arterial duplex to document the distribution of disease prior to recruitment and subjects with isolated iliac disease suitable for angioplasty or an ankle-brachial pressure index of greater than 0.9 were excluded from the study. In view of the need for skeletal muscle biopsy subjects with evidence of critical ischaemia manifest by rest pain or tissue loss and subjects on oral anti-coagulants were excluded from the study.

Exercise regime

A twelve-week supervised exercise-training programme was utilised. The exercise classes were established in 2001 and a randomised control trial has previously demonstrated this regime to be effective.[17] The training exercises, delivered in a circuit format, were targeted at the calf muscles. Following a 10 min warm up of passive stretching exercises, each of the five exercise stations was undertaken for 8 min. The exercises were designed to require little or no specialist equipment such that patients could continue the programme in their own home and treadmill exercise was deliberately excluded in order to prevent familiarisation bias. The exercises comprised; step ups, toe walking, heel raises, wobble board and resistance cycling. As exercise to claudication has been identified as a key factor in success, patients are encouraged to exercise to the point of claudication.[9]

Treadmill assessment

Subjects' maximum walking distance (MWD) and claudication distance (CD) were recorded using a fixed ramped treadmill assessment at 2.5 km/kph at a 10% incline, as this proved tolerable and reproduced claudication symptoms in the majority of patients. All subjects underwent treadmill assessment at recruitment and upon completion of the study. The tests were undertaken on the same Trimline 4000 treadmill. The display was shielded from the patients view, such that the time and distances walked could be kept from the patient in order to prevent bias.

Muscle biopsy

Biopsies were taken at a separate appointment from the treadmill testing. Subjects were brought to the department by car or taxi and biopsies taken after a 30-min period of rest in order to prevent any confounding effects of acute exercise. Biopsies were taken with a modified Bergstrom needle technique.[18] Following injection of between 2 and 5 ml of plain 2% lidocaine, the skin and muscle fascia were incised through a horizontal stab incision. The subsequent biopsy was taken 15 mm adjacent to the first in order to minimise differences resulting from muscle heterogeneity, but in order to be sufficiently separated to prevent biopsying scar tissue. The specimens were labelled with a sequentially allocated patient number which did not elucidate in which arm of the study the

subject was involved and were then snap frozen in isopentane cooled by liquid nitrogen and stored at −80 °c for subsequent batch homogenization. Sample analysis was not truly blinded as patient recruitment, treadmill visits, muscle biopsy and sample processing were all undertaken by the lead investigator.

Sample processing

Samples were subsequently defrosted and mechanically homogenized in lysis buffer (containing 10 mM Tris HCl, 5 mM EDTA, 30 mM NaCL. 50 mM NaF, 100 μM sodium orthovanadate, 1% Triton X-100, 01 mM PMSF, phosphatase inhibitor (sigma P5726) and protease inhibitor cocktails (sigma −P8340)). The samples were then centrifuged for 15 min at 4 °c at 1000g. Following centrifugation the supernatant protein concentration was determined by bicinchoninic acid (BCA) assay and 50 μg of protein per well was suspended in sample buffer and loaded onto the polyacrylamide gels in duplicate. 8% resolving gels were used[19,20] and the gels were run in a cold room overnight at 16 mA. The position of the protein bands was confirmed against 20 μL of Biorad colour marker RPN 756V. In view of the difficulties of reliably transferring such a large protein to a membrane for Western blotting, quantification of the three MHC bands was achieved on gel by optical density analysis following silver-staining Fig. 1, as had been described by Keteyian.[16] The relative expression of the three MHC isoforms could then be determined as a proportion of the total optical density.

Data analysis

The relative proportions of MHC I, IIa and IIb are expressed as percentages of the total. Statistical analysis using SPSS, comparisons made between groups for normally distributed variables by independent samples t testing and within groups by paired sample t testing. Non-parametric comparisons between groups by Mann Whitney *U* test and within groups by Wilcoxon signed rank. Non-parametric data is presented as median with the (interquartile range) and parametric as the mean ± the standard deviation.

Results

The demographic data for the study and control groups is presented in Table 1. At recruitment there was no significant difference in age, sex, resting ankle brachial pressure, smoking status, comorbidity or treadmill assessed maximum walking distances between the two groups. The distribution of disease was similar between the two groups (Table 2) with predominantly superficial femoral artery disease in both, it should be noted however that there were 3 patients in the exercise but not the angioplasty group with common femoral artery stenoses. Upon completion of the supervised exercise programme a significant 94% increase was witnessed in the maximum walking distance (241 (193–265) metres, $p < 0.05$). No such improvements were witnessed in the control group following the period of exercise advice (131 (114–152) metres, $p > 0.20$).

The relative expression of the myosin heavy chains is shown in Table 3.

A significant 11.1% ($p = 0.02$) increase in the proportion of slow oxidative MHC I expression was observed upon completion of the supervised exercise programme, which did not occur in the control group. This was accompanied by corresponding reductions in the proportion of MHC IIa and IIb, although neither reached independent significance. Similarly there was no significant correlation between the level of MHC I expression and the treadmill assessed walking distances ($r = 0.58$ $p > 0.1$). There was however, a significant positive correlation between the subjects' percentage increase in claudication distance and the change in MHC I expression upon completion of the supervised exercise-training programme ($r = 0.69$ $p < 0.05$). When the data is analysed by dividing the subjects into quartiles based upon their percentage change in MHC I expression, (Table 4) it can be seen that upon completion of the supervised exercise programme those subjects in whom the greatest increment in MHC I expression was witnessed were also those in whom the greatest increases in both claudication and maximum walking distances were witnessed.

In view of previous data[16] suggesting a differential response in MHC expression between the sexes, comparisons were made between the men and women in the exercise study (Table 5). The majority of subjects recruited to the study were male (20:7). There was no significant difference in the proportion of MHC type I expression at recruitment when compared between men and women ($p = 0.60$). On completion of the exercise programme

Table 1
Group demographics at recruitment.

	Exercise group	Angioplasty group
	$n = 27$	$n = 11$
Age		
Mean ± SD	67.6 ± 6.1	68.3 ± 5.8**
years		
Sex M:F	20:7	8:3*
ABPI	0.66 (0.55–0.79)	0.68 (0.54–0.82)*
median (IQR)		
Current Smoker	9 (33%)	3 (27%)*
Diabetes	10 (37%)	4 (36%)*
MWD metres	124 (106–145)	137 (103–161)*

At recruitment there were no significant differences in the age, resting ankle brachial pressure indices or the treadmill-assessed maximum walking distances between the two groups. ***p > 0.50** ****p > 0.20**.

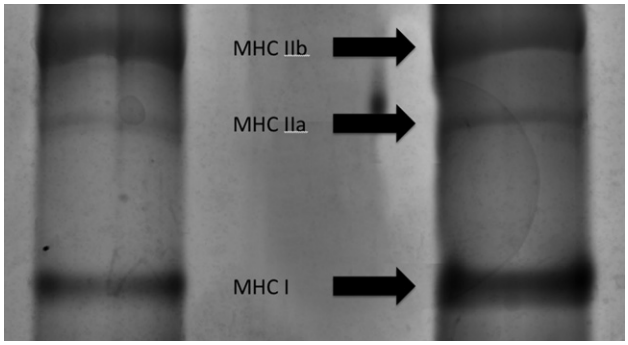

Figure 1. A digital scan of a silver stained 8% resolving gel demonstrating the three myosin heavy chain bands. This gel shows paired pre and post intervention muscle biopsies from a subject in the exercise group.

Table 2
Anatomical distribution of arterial disease at recruitment.

Duplex detected disease	Exercise group	Angioplasty group
	$n = 27$	$n = 11$
Iliac	3	3
Common femoral	3	0
Superficial femoral	21	10
Popliteal	6	1
Crural	2	0
Disease at more than one level	8	3

Table 3
Myosin heavy chain expression by group.

Proportion of MHC expression		% MHC I	% MHC IIa	% MHC IIb
Exercise group	Recruitment	34.3 ± 6.8	42.0 ± 7.1	23.7 ± 4.7
	Completion	45.4 ± 4.4*	36.7 ± 4.9	17.9 ± 4.2
Angioplasty group	Recruitment	36.1 ± 5.9	41.1 ± 5.1	23.8 ± 4.1
	Completion	37.4 ± 5.7	38.7 ± 5.1	23.9 ± 5.1

Proportion of Myosin heavy chain I, IIa and IIb expression as a percentage of the total for each biopsy. There was a significant increase in the proportion of MHC I expression in the exercise group. $^{*}\boldsymbol{p = 0.02}$.

significant increases in MHC I expression were witnessed in both men and women (13% and 9% respectively).

Discussion

This study has demonstrated that supervised exercise training in claudicants is associated with a significant increase in the proportion of slow oxidative myosin heavy chain type I expression within the symptomatic gastrocnemius muscle. Increases in the slow oxidative MHC I are potentially beneficial for claudicants, by increasing oxidative capacity and reducing the rapid switch to anaerobic glycolysis associated with faster glycolytic fibres, thereby reducing the painful accumulation of lactate. There is in addition evidence using both whole body calorimetry and 31P-MRS[21,22] that an increase in type I muscle fibres and a reduction in type IIb fibres is associated with increased metabolic economy.

This data contradicts a previous report in which immunohistochemistry was used to determine muscle fibre type.[14] In his small study Hiatt reported that a twelve-week treadmill based supervised exercise programme was associated with both improved treadmill assessed walking distances and increased peak oxygen consumption, benefits that were not witnessed as a result of strength training. However, when gastrocnemius biopsies were taken from these 10 subjects, despite the significant symptomatic improvement, the proportion of type I myofibres in the symptomatic limb, determined by area, was unchanged. This apparent discrepancy is likely to result from the use of mATPase immunohistochemistry, given the evidence that muscle fibres are actually hybrids, expressing more than one myosin heavy chain isoform along their length and that the proportion of hybrid fibres is increased both by exercise and age.[15,23] It is likely therefore, that Hiatt's results reflect a tendency of traditional mATPase immunohistochemistry, used for fibre typing, to underestimate the degree of myosin heavy chain adaptation.

One previous report has used a combination of both mATPase immunohistochemistry and MHC electrophoresis to seek evidence of skeletal muscle adaptation in response to progressive resistance training in claudicants.[24] In contrast to previous data[14] McGuigan reported that progressive strength training could produce significant improvements in walking performance. He observed increases in the overall type I and type II muscle fibre areas, as determined by mATPase immunohistochemistry. In addition McGuigan found that strength training was associated with a reduction in MHC IIb expression measured by SDS-PAGE electrophoresis.

McGuigan found no significant change in the percentage of MHC I or MHC IIA. Given that the relative proportion of type I fibres has been shown to correlate with exercise performance in patients with peripheral vascular disease,[25] these findings are surprising. However the apparent discrepancy is likely to result from the specific form of progressive resistance training used. Certainly in healthy individuals the differential effects of strength versus aerobic exercise training are well reported.[26]

In this study, there was no correlation between the absolute proportion of MHC I expression and treadmill assessed walking performance, a finding which contradicts data published by Askew in 2005.[25] There was however a significant positive correlation between the percentage increase in MHC I expression and the subjects improvement in their treadmill assessed claudication distance ($r = 0.69$, $p < 0.05$).

One previous report of exercise training in heart failure patients has suggested that there may be sex differences in the responses of skeletal muscle to exercise training.[16] In this study there was also a male predominance (20 men, 7 women), however when both recruitment myosin heavy chain expression and the exercise response were compared, there was no difference between the men and women, with increases in the proportion of type I myosin heavy chain of +13% and +9% respectively. Keteyians' study was clearly flawed as a result of; the small sample size, absence of a control group and the variability of any particular subjects duration of training (14–24 weeks). Nevertheless, one alternative explanation for this difference is that exercise training in the presence of claudication may be a more potent stimulus for local skeletal muscle adaptation than exercise training in the presence of heart failure. Certainly models, in which unilateral exercise has been undertaken in the presence of ischaemia, have shown that it is a more potent stimulus of growth factor expression.[27]

The plasticity of human skeletal muscle in response to exercise training has been extensively studied in health and the observation that endurance athletes have a predominance of MHC Ia as compared to the predominance of MHC IIa/IIx seen in sprinters[28] is evidence that differing exercise regimes can have quite different effects upon muscle structure. There must therefore be differing signalling pathways for muscle hypertrophy in response to resistance training and muscle metabolic adaptation in response to endurance training. The mammalian Target of Rapamycin (MTor), a member of the phosphatidylinositol kinase family, appears to be responsible for the muscle hypertrophy seen following strength training,[29] whereas muscle metabolic adaptation appears to be partly an activity dependent phenomenon in which calcium influx is responsible for activation of the calcineurin-NFAt pathway (nuclear factor of activated T cells). This has been shown amongst

Table 4
Percentage changes in MHC I expression and treadmill walking distances upon completion of the exercise programme.

MHC I expression increment by quartile	Percentage increase in Maximum Walking Distance upon completion			Percentage increase in claudication distance upon completion		
MHC I	*n*=	Median	Range	*n*=	Median	Range
+ 0–4%	3	53%	(38–76)	2	68%	(54–82)
+ 4–8%	5	77%	(65–103)	5	89%	(61–112)
+ 8–12%	12	109%	(47–171)	14	114%	(34–163)
+ 12–16%	7	163%*	(131–322)	6	156%*	(117–241)

A table comparing the percentage increase in treadmill assessed walking distances upon completion of the exercise trial with the increase in MHC I expression divided into four quartiles. $^{*}\boldsymbol{p < 0.05}$ Kruskal Wallis.

Table 5
Gender differences in myosin heavy chain expression within the exercise group.

Exercise Group	Men $n = 20$		Women $n = 7$	
	Recruitment	Completion	Recruitment	Completion
% MHC I	33.9 ± 6.8	46.9 ± 5.9*	35.2 ± 4.8	44.2 ± 3.9*
% MHC IIa	42.5 ± 6.1	37.2 ± 4.6	39.7 ± 7.3	34.1 ± 5.1
% MHC IIb	23.6 ± 4.6	16.9 ± 4.9	25.1 ± 4.9	21.7 ± 3.6

Subgroup analysis of myosin heavy chain expression by sex, revealed that following supervised exercise there was significant increase in the proportion of MHC I for both men and women. $^{*}\boldsymbol{p < 0.05}$.

other effects, to lead to increased expression of the slow MHC I isoform.[30] There is also evidence that local hypoxia is a potent stimulus to skeletal muscle adaptation. It is known that the greatest improvements in walking distances are associated with exercise beyond subject's claudication distance and there is certainly good evidence that it is a potent activator of growth factor expression.[27] In addition the greatest improvements in walking distances are associated with exercise beyond subject's claudication distance and hypoxia may be the most potent stimulus in claudication. Hypoxic ischaemic factor (HIF) is a key signalling mechanism, which has been linked to increased vascular endothelial growth factor (VEGF) and Peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) expression. HIF is post transcriptionally regulated, being stable in hypoxic conditions and stable analogues of HIF may therefore offer therapeutic applications. There is certainly evidence that single bouts of exercise are associated with increased vascular endothelial growth factor expression[31] and these may be maintained following several weeks of exercise training.

The authors accept the limitations associated with an unblinded and non-randomised study. This may introduce bias particularly with regard to any difference in the distribution of disease between the exercise and control group. There were however no differences in MHC expression between the two groups at recruitment in this study. Whilst progressive changes in MHC expression have previously been described in patients with deteriorating peripheral arterial disease, these changes were limited to subjects with critical ischaemia and as such are unlikely to have had any bearing upon the results of this study.

This study has demonstrated that a circuit-based programme of non-treadmill exercises is an effective symptomatic treatment for claudication producing significant improvements in walking distances. In addition it is the first report in which supervised exercise training for claudication has been shown to increase the relative expression of slow oxidative myosin heavy chain type I. Whilst it has been reported that some of the benefits witnessed following exercise for claudication are systemic,[31] this fibre type adaptation resulting in enhanced metabolic economy, suggests that local skeletal muscle adaptation may be a key event.

Conflict of Interest

None.

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